AN ANALYTICAL STUDY ON GENETIC VARIABILITY AND HYDROGEN (H_2) PRODUCTION FROM CYANOBACTERIA

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ABSTRACT

This research paper entitles the research work regarding bio-hydrogen generation from a renewable source, namely biomass; such as a group of photosynthetic micro-organisms cyanobacteria. Molecular hydrogen is widely regarded as the energy carrier of the future. It can be used in fuel cells, generating an electric current. Bio-hydrogen production has several advantages when compared to photo-electrochemical or thermo-chemical processes due to the low energy requirement and investment cost. Cyanobacteria has an inherent ability to produce molecular hydrogen via the enzyme complex nitrogenase. This hydrogen is not released, however but is recaptured by the bacteria using an uptake hydrogenase. The study also foretells about optimization of the various key parameters that enhance hydrogen production. This knowledge can be used to evaluate the possibilities for producing hydrogen and high value products efficiently in the same process.

Keywords: Hydrogen Generation, Biomass, Dark Fermentation, Photosynthesis, Hydrogen Energy, Molecular Hydrogen, Bio-Hydrogen, Nitrogenase, Hydrogenase.

INTRODUCTION

The interaction between Earth and Human systems is more intense than ever before due to an accelerated socio economic development involving population growth, technology, trade, production, consumption patterns, and governance among others. As a consequence our natural resources on Earth are being rapidly consumed; varied land use practices are employed across the world as a result of urban sprawl while food security, desertification, soil erosion, degradation, looming consumption and exhaustion of our fossil fuel reserves to produce ~90% of the energy that we need are contemporary and increasing problems.

Three major interconnected problems that seriously threat our world and civilization arise from this global scenario:

- ✓ Climate change due to accumulation of greenhouse gases in the atmosphere,
- ✓ Waste disposal and the need of renewable;
- ✓ Environment friendly sources of energy.

In addition energy demand is increasing rapidly according to world population which is estimated to grow from 6 to 9 billion by 2050, number of light-duty vehicles is expected to increase from 1 to 2.8 billion cars by 2050 and highly populated developing countries will become more energy intensive as they expand their economies.

The concept of sustainable development was evolved for a liveable future where human needs are met while keeping the balance with nature. The world's energy requirements were being fulfilled by fossil fuels which serve as a primary energy source. Hence, the overwhelming scientific evidence was that the unfettered use of fossil fuels has caused the world's climate to change, with potential disastrous effect. Moreover, the oil crises, which surfaced during 1973, provided a reminder that breaking an energy paradigm based on fossil fuel dependency would lead to economic and environmental advantages.

Hydrogen holds a promise as a potential clean, renewable and environmental friendly energy source. Currently 95 to 99% of hydrogen are produced from fossil fuel. The classical methods of producing hydrogen include steam reforming of natural gases, coal gasification and electrolysis of water. Conventional hydrogen gas production methods are energy intensive processes requiring high temperatures (>840^0 C) and not environmental friendly. Electrolysis of water, although the cleanest technology for hydrogen gas production, can only be used in areas where electricity is cheap because electricity accounts for 80% of the operating cost of H_2 production. Recent research on hydrogen indicated that the worldwide need for hydrogen is increasing with a growth rate of nearly 12% per year for the time being and contribution of hydrogen to total energy market will be 8-10% by 2025.

A sustained program of research and development into many areas of hydrogen as energy carrier was started during the year 1977, with the initiation of the International Energy Agency. In spite of this international interest, a combination of continued availability of cheap oil and nuclear energy stalled the wider acceptance of hydrogen energy until recently. Hydrogen is strategically important as it has low emission, is environmentally benign, and represents a cleaner and more sustainable energy system.

In the present time, the interests of scientists are turned to truly inexhaustible raw materials for biofuel production including water for hydrogen production by its biophotolysis or photocatalytic decomposition. In the field of biofuels, hydrogen is the purest and most valuable of the produced fuels and may be the most promising candidate for the role of an environmentally friendly and renewable energy carrier of the future.

HYDROGEN AS AN ENERGY CARRIER

Hydrogen (H_2) is the most abundant element in the universe and the third most abundant element on Earth. Under ordinary conditions H_2 is an invisible, nontoxic light gas that is very rare in the atmosphere (~ 1 ppm by volume). Because of its high reactivity H_2 is always combined with other elements; it is present in water, hydrocarbons, in every living organism and in natural and artificial compounds.

The energy content of H_2 (285.9 KJ.mol-1) is 2.7 times higher than the energy content of gasoline and, when used in fuel cells, the combination of H_2 and oxygen (O_2) generates electricity, heat and water. The efficiency of a fuel cell to produce power or electricity is not limited by the Carnot cycle as the case for fossil fuels (which is ~27 % efficient for most automotive engines); it is determined by the ratio of the free energy change ($\Delta G°$) and the enthalpy change ($\Delta H°$) of the chemical reaction between H_2 and O_2, typical efficiencies of hydrogen fuel cells are between 50-70%. This implies that the efficiency of a H_2 fuel cell vehicle is 2-3 times greater than that of conventional gasoline vehicle and 1.5-2 times greater than diesel-electric hybrids. Based on these considerations H_2-fuel cell systems constitute a solution for oil saving and power generation in the transport sector.

Molecular hydrogen is widely regarded as the energy carrier of the future. It can be used in fuel cells, generating an electric current. The electricity generated can be employed to drive an engine, and fuel cell engines are thought to be a future replacement for combustion of engines. The main benefit of this type of engine is that the only exhaust fume is water. In order to make use of hydrogen powered fuel cells a real environmental advantage, the hydrogen used should come from a renewable source, and no pollution should be generated in the production. For this, hydrogen must be produced from a biological system such as filamentous cyanobacteria which can be grown in bioreactors.

TYPES OF BIOHYDROGEN PRODUCTION

Bio-hydrogen production can be achieved by two kinds of biological processes

- Light Dependent
- Light Independent

Light Dependent H_2 Production	Light Independent H_2 Production
1. Photolysis **2. Photofermentation**	Dark Fermentation

ADVANTANGES OF BIOLOGICAL PROCESSES FOR HYDROGEN PRODUCTION

- Simplicity in operation
- Wide availability of renewable feed stocks
- Carbon neutrality
- Cost-effectiveness in operation

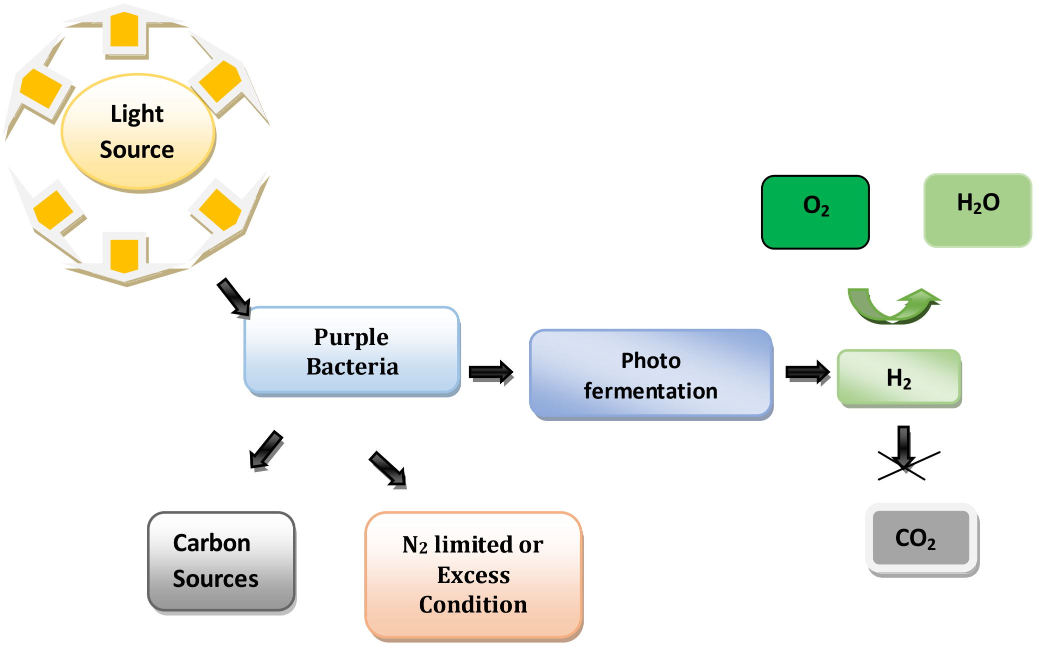

Figure 1: A Schematic Representation of Light Dependent H_2 Production

BIOLOGICAL METHODS OF HYDROGEN PRODUCTION

The worldwide hydrogen production from fossil energy resources is near about 95%. Many microorganisms contain enzymes, known as hydrogenases that either oxidize hydrogen to protons and electrons or reduce protons and thus release molecular hydrogen. Most of the biologically produced hydrogen in the biosphere is derived from microbial fermentation processes. These organisms decompose organic matter to carbon dioxide and hydrogen. Hydrogen producing bacteria can grow under autotrophic conditions with hydrogen gas as the sole reducing power and energy substrate. In these bacteria, oxygen serves as a terminal electron acceptor leading to water as the end product.

The photobiological hydrogen production process uses microorganisms and sunlight to turn water, and sometimes organic matter, into hydrogen. This is a longer-term technology pathway in the early stages of research that has a long-term potential for sustainable hydrogen production with low environmental impact.

Biological hydrogen production may be achieved by various microorganisms under distinct environmental parameters (see Chart 1); it is mainly classified into as follows:-

- ❖ Direct photolysis by green microalgae,
- ❖ Indirect photolysis by cyanobacteria,
- ❖ Photo fermentation by photosynthetic bacteria (PSB), and
- ❖ Dark fermentation by facultative anaerobe

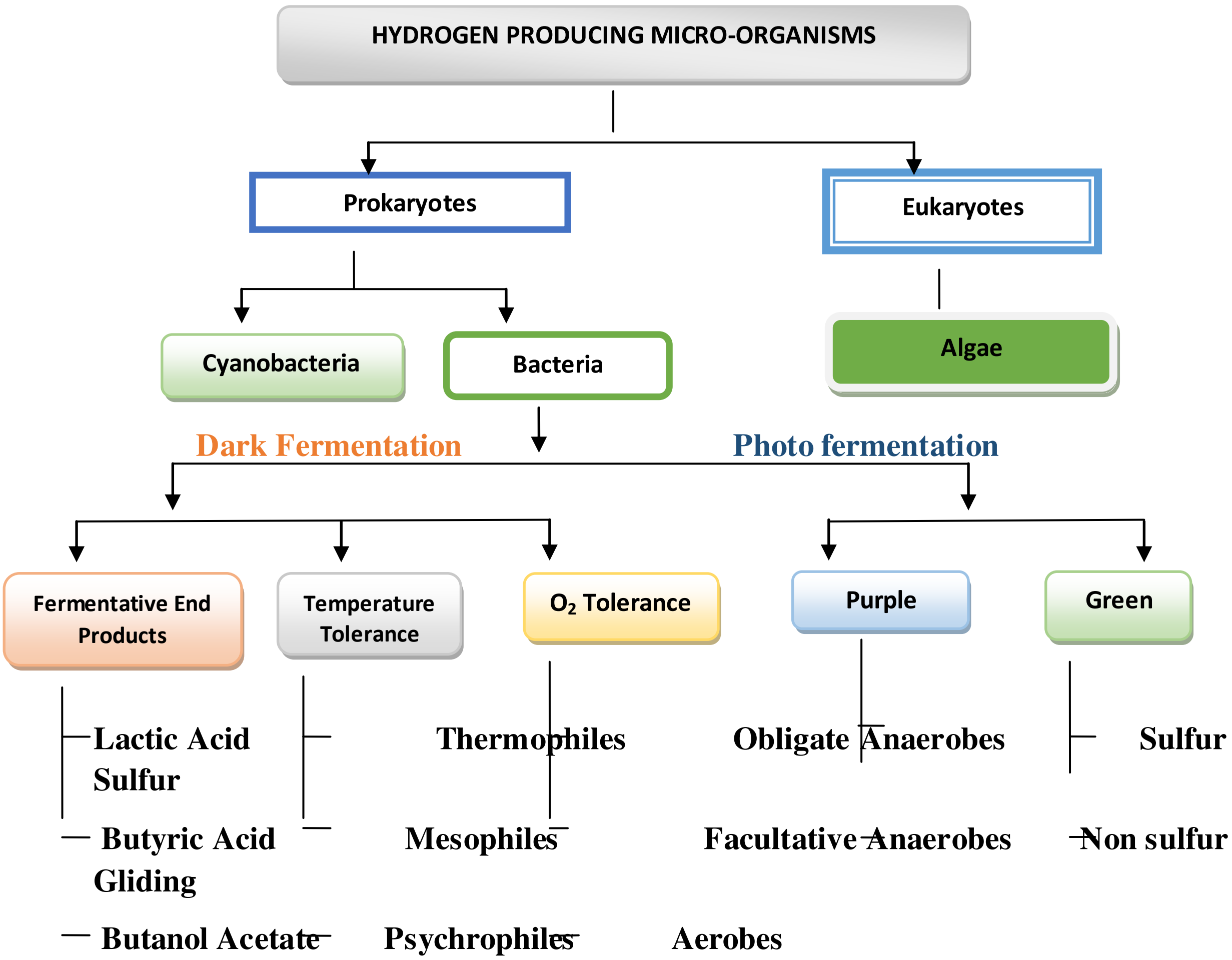

Chart 1: A Summary of H_2 Producing Micro-Organisms

CYANOBACTERIA AS A SOURCE OF BIOMASS

Cyanobacteria are an ancient and widespread group of microorganisms, belonging to the eubacteria. They are found in almost all environments on Earth. Some of them are found on the surface of glaciers, some live inside rocks in hot deserts, and yet others in hot springs. They have been found in fossil form in rock as old as 3.5 billion years.

Cyanobacteria come in many forms. They can be unicellular or form filaments of cells connected to each other. These filaments can be found individually or in colonies of different shapes. They can fix atmospheric nitrogen using nitrogenase enzyme to reduce nitrogen (N_2) into ammonia. Since nitrogenase is an oxygen sensitive enzyme, nitrogen fixing cyanobacteria have evolved a variety of strategies to combine nitrogen fixation with oxygen-evolving photosynthesis.

Cyanobacteria are photosynthetic organisms that are suitable for photobiological hydrogen yield. They have an ability to capture light energy and convert it to chemical energy. Few cyanobacteria are capable of producing hydrogen, yet they do not naturally release it from their cells. If cyanobacteria can be made to efficiently evolve hydrogen using energy from sunlight, they could be used as a source of clean.

Some cyanobacteria belonging to the genera *Nostoc*, *Anabaena*, *Synechocystis*, *Oscillatoria*, *Synechococcus*, and *Phormidium* contain the enzyme hydrogenase used to produce hydrogen. They can grow on simple nutrient media due to their ability to absorb CO_2 from the atmosphere as a source of carbon, and many strains can reduce the atmospheric N_2 to ammonia, moreover, they can use sunlight as their only source of energy for hydrogen production. Unique features of cyanobacteria include their ability to survive under extreme conditions, photosynthesis involving the participation of unusual pigments, nitrogen fixation, the release of various compounds into cultural media, and a specific relation with oxygen are determined by the specific structure of a protoplast, the metabolism, plasticity, and the unique enzymatic system of their cells.

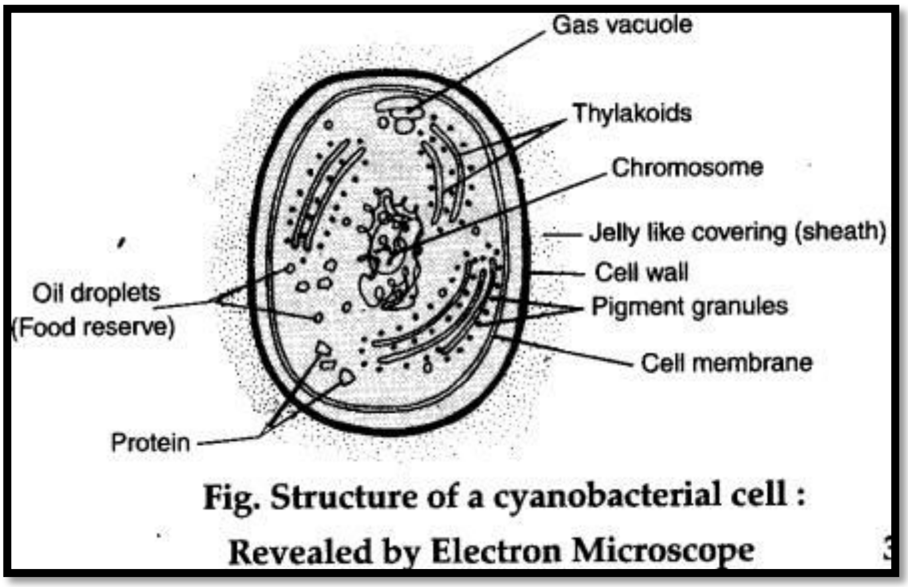

Figure 2: Structure of Cyanobacteria Revealed Under An Electron Microscope

BIOCHEMICAL PRODUCTION OF HYDROGEN (H_2) GAS FROM CYANOBACTERIA

Many research and experimental works are devoted to the processes of hydrogen formation due to the conversion of solar energy by cyanobacteria cells. Morphological and metabolic features make them the most promising hydrogen producers. Most studies focus on the metabolism characteristics of heterocystic forms of cyanobacteria, which provide light-dependent hydrogen release due to spatial separation of hydrogen and oxygen release processes.

Metabolic Enzymes Possessed By Cyanobacteria

Hydrogen Metabolizing Enzymes

- **Nitrogenase**
- **Membrane Bound Hydrogenase**
- **Soluble Hydrogenase**

Cyanobacteria, in general, possess three hydrogen-metabolizing enzymes: nitrogenase, membrane bound hydrogenase, and soluble hydrogenase. The hydrogenases utilize the hydrogen, whereas nitrogenase maximizes the hydrogen generation.

The detection of hydrogen metabolism enzymes in single-cell nitrogen-non-fixing cyanobacteria is interesting and attracts the attention of many investigators. Cyanobacteria are promising biological systems for solar energy transformation. Furthermore, cyanobacteria, due to their unique and labile pigment composition, absorb light in a wide range of wavelengths, and their ability to store light energy can be sufficiently high. In general, the potential rate of hydrogen release can be comparable to the rate of photosynthesis.

However, although they are promising biological systems for solar energy conversion, there are many limitations, such as oxygen sensitivity of basic hydrogen metabolism enzymes, instability of the hydrogen release process, etc. Therefore, presently the production of hydrogen based on cyanobacteria is still at the initial stage of its development within laboratory, and pilot plants.

Biochemical production of hydrogen as a by-product of the metabolism of microorganisms is a relatively new area of technological development, and a promising renewable energy source for the future. Since the possibility of such reactions, occurring in living organisms was demonstrated more than half a century ago, the process of biophotolysis conducted by cyanobacteria has been actively studied over the past 35 years.

The basic molecular mechanisms of hydrogen take up by living systems were studied. Systems of water biophotolysis, which have two common elements, such as the electron transport chain of photosynthesis, including a water decomposition system and hydrogen formation catalysts, are conceptually considered as direct and indirect biophotolysis.

The process of direct biophotolysis involves the use of light energy of absorbed by the photosynthetic apparatus for the water cleavage with the formation of oxygen and for the generation of low-potential reducing agents followed by the reduction of protons and the production of hydrogen. During indirect biophotolysis, the products of photosynthetic water cleavage and the products of subsequent reduction of ferredoxin are used to fix carbon dioxide, and the resulting reduced carbon compound can be used to stimulate hydrogen release in a separate reaction.

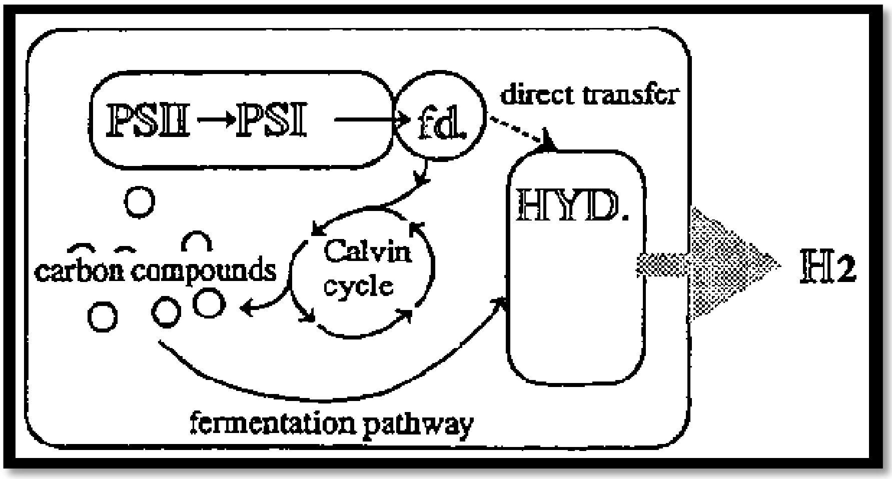

Figure 3: Hydrogenase Mediated H_2 Production in Cyanobacteria

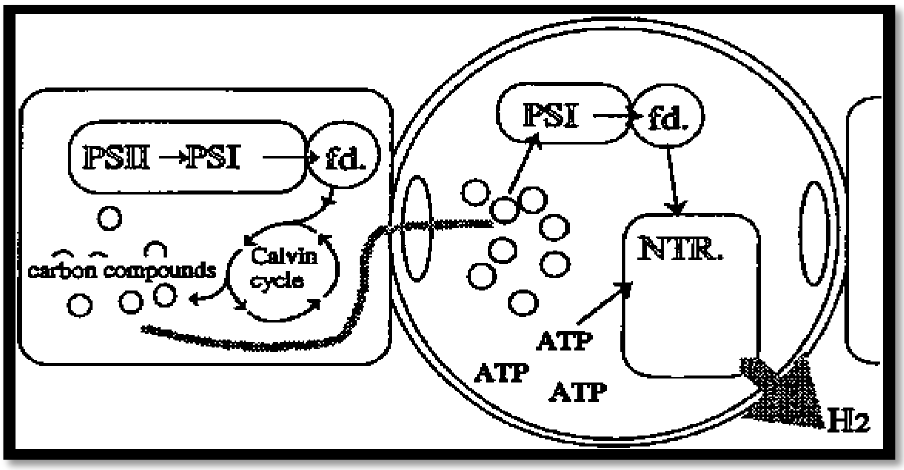

Figure 4: Nitrogenase Mediated H_2 Production in Cyanobacteria

MATERIALS AND METHOD

- **Strains and Growth Conditions**

Strain collection was maintained photo-heterotrophically in flasks with BG11 media, provided with low light intensity (about 20 μmol photons m^{-2} s^{-1}) by means of cool white lamps at pH 7.2 ± 0.1 and temperature 25 ± 1 °C.

Pigment analysis and Growth Studies A fixed amount of the homogenous culture was taken for chlorophyll and carotenoid analysis with respect to control. Chlorophyll and carotenoid pigments were extracted in methanol. The absorption was measured at 660 nm for Chlorophyll a and 480 nm for carotenoid. OD was recorded for growth at 750 nm for 0th hour, 24th, 48th, 72th and 90th hour.

- **Physiological Analysis of Algal and Cyanobacterial Cells**

Pulse Amplitude Modulated (PAM) Fluorometer Configuration and Measurement Procedure In our experiments. Chlorophyll a fluorescence induction was measured with pulse amplitude modulated (PAM) fluorometer (Dual-Modulation Kinetic Fluorometer, Photon Systems Instruments, Czech Republic) to provide saturating flashes.

- **Medium Preparation**
- **ASP2** Stock solutions of ASP2 salts, iron solution, buffer solution, and P1 metal solution were prepared first. Preparations of ASP2 salts were simply done by dissolving disodium ethylenediamine tetraacetate (Na_2EDTA), potassium chloride (KCl), calcium chloride dehydrate ($CaCl_2$ $2H_2O$) and potassium phosphate dibasic (K_2HPO_4) in deionised. Iron solution was prepared from iron (III) chloride hexahydrate ($FeCl_3$ $6H_2O$) and hydrochloric acid (HCL) dissolved in deionised water.
- Buffer solution is a buffer solution prepared from TAPS ($C_7H_{17}NO_6S$) and TAPSO ($C_7H_{17}NO_7S$) salts dissolved in deionised water includes ASP2 salts, iron solution, buffer solution, P1 metal solution, sodium chloride (NaCl) and magnesium sulphate ($MgSO_4$). The addition of NaCl and $MgSO_4$ is to increase the salinity of ASP2 medium in order to match that of actual sea water.

RESULTS AND DISCUSSION

Logistic Growth Model

A logistic growth function, Equation is a common model of population growth, first published by Pierre-Francois Verhulst in 1838, to describe the self-limiting growth of a biological population. It has been frequently and effectively used to fit kinetic parameters to algal growth and nutrient uptake data. In this equation, X_{max} represents the maximum dry biomass concentration (g L^{-1}) obtained in a particular experiment. μ_{max} is the maximum specific growth rate (hr^{-1}), which describes the number of cell divisions per unit time under the respective condition. t_0 is the inflexion point of the function (hr). X(t) is the dry biomass concentration at any specific time, t. A clear representation of individual logistic growth model's parameter can be seen from Figure 5. The gradient of the microbial growth profile at $t = t_0$ is considered as the maximum specific growth rate.

$$X(t) = \frac{X_{max}}{1+e^{-\mu_{max}}} \times (t-t_0)$$

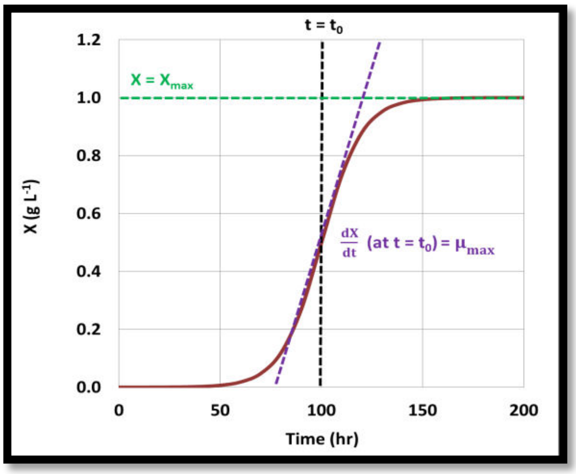

Figure 5: Representation of Logistic Model's Parameters

The first "lag phase" is the time during which microorganisms need to adapt themselves to the new cultivating environment after inoculation. As a result, during this period, a minimal change in the biomass concentration is observed for the first 20 hours. Following, the secondary "exponential growth phase" is clearly marked between 20 - 70 hour, during which simultaneous increase in the density of *Cyanothece* 51142 culture, from 0.2 to 2.4 g L^{-1}, and decrease in an available nitrate concentration, from 1244 to 100 mg $/NO_3$ /L, are clearly seen. During this period, the rate of cyanobacterial reproduction proceeds at its maximum value, μ_{max}, which is an exponential function of time, as long as the cells have access to sufficient illumination and nutrients.

Figure 6: Hydrogen Production From Biomass

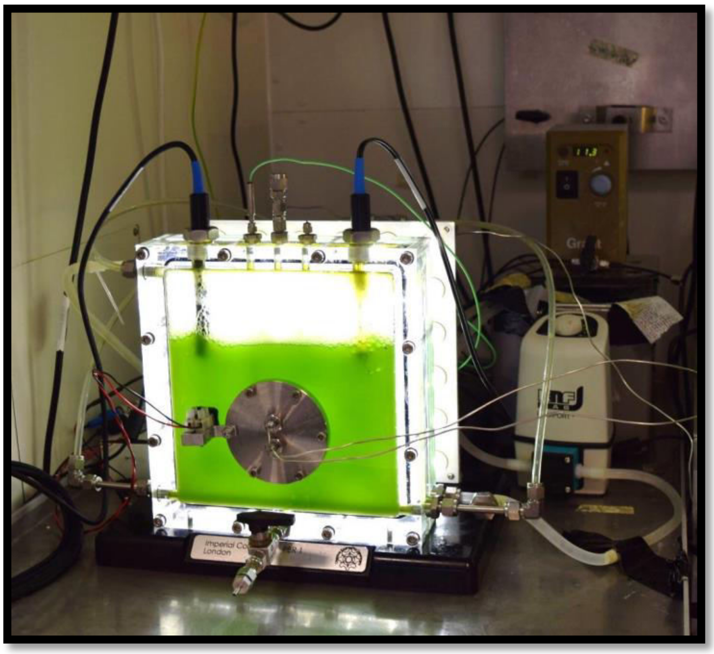

Figure 7: Experimental Set-Up of The ICL Flat-Plate PBR At Department of Chemical Engineering

CONCLUSION

Biological hydrogen production is one of the most challenging areas in biotechnology, with respect to environmental and energy-source problems. In the past decade, hydrogen energy has progressed on all fronts, making in road into all areas of energy. The concept of bio-hydrogen generation is based on the exploitation of bacteria to produce hydrogen as a by-product during the growth on biomass. Existing technologies offer high potential for the development of practical H_2 production bioprocesses. Further research and development aimed at increasing rates of synthesis and final yields of H_2 are essential. Bioprocess integration, optimization of bioreactor design, rapid removal and purification of hydrogen and especially, directed evolution of hydrogenase and metabolic engineering of the H_2-evolving microorganism offer exciting prospects for biohydrogen systems, and some novel strategies will also be very encouraging and exciting in the future. The rapid advances of biological and engineering sciences will greatly facilitate the overcoming of existing bottlenecks as well as new challenges and create new opportunities for economical hydrogen production in the near future.

FUTURE PROSPECTS

The future of biological hydrogen production depends not only on research advances, *i.e.* improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems.

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I pay tribute to My Parents for lifting me up till this phase of life. I thank them for their love, trust, patience, support and bearing all kind of stress to make me what I am.

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